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Effects of Temperature on Tissue Thermal Injury and Wound Strength After Photothermal Wound Closure

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Background and Objectives: **Our goal was to determine the effect of temperature on the induction of tissue damage after laser-welded wound closure with and without albumin solder.**
Study Design/Materials and Methods: **Multiple full-thickness skin incisions were made in a porcine model. Incisions were repaired by using a 1.32-μm laser at temperatures of 65°C, 75°C, 85°C, or 95°C with and without a 50% human albumin solder. The rate of apoptosis (programmed cell death) was quantified by counting the proportion of cells that stained positively for nuclear DNA fragmentation (nick end labeling). The distance that necrosis extended from the wound edge was also measured. The strength of the weld was measured with a tensiometer.**
Results: **For laser-welded repairs with solder, the amount of apoptosis at 65°C and 75°C was comparable to that of controls but became significantly elevated at 85°C and 95°C. The extent of necrosis was similar to that of controls at low temperature but also increased at 95°C. Incisions repaired without solder showed increased necrosis compared with those repaired with solder at temperatures of 65°C, 75°C, and 95°C at 0–0.5 mm from the incision. Wounds repaired at 85°C and 95°C showed more apoptosis in the absence of solder. The increased cell death at higher temperatures correlated with significantly decreased wound strengths at 3 days after repair in the solder group. A lower rate of cell death was observed in the solder group, which correlated with superior wound strength when compared with repairs without solder at days 0 (65–95°C) and 3 (95°C).**
Conclusion: **Both apoptotic and necrotic cell death were used as quantitative measures of tissue injury and were accurate predictors of short-term wound strength. The addition of albumin solder decreased overall tissue injury. These results suggest that temperatures of 65–75°C with solder provide the optimal conditions for maximizing acute wound strength and minimizing tissue injury. Lasers Surg. Med. 25:285–290, 1999.**

Key words: albumin; apoptosis; necrosis; nick end labeling

INTRODUCTION

Several factors have been shown to affect wound strength after laser tissue welding. These

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factors include the use of a tissue solder, the composition of the solder, and the temperature of the tissue surface [1].

Previous work has shown that acute and chronic skin wound strengths are highly dependent on the tissue temperature during laser photocoagulation, presumably related to the degree of thermally induced tissue injury [2]. Standardization of the welding process is accomplished by the addition of a temperature-controlled laser photocoagulation system (TCPC). With the TCPC system, it is possible to control the surface tissue temperature accurately during laser photocoagulation. The TCPC system enables us to evaluate the effects of temperature more accurately by maintaining the tissue surface temperature within a constant range [3]. Although the effect of temperature and solder on welded tissue can be studied by examining wound strength, the extent and mode of cellular injury induced by laser welding remain unclear.

Two distinct types of cell death are involved in tissue injury: necrosis and apoptosis (programmed cell death) [4–6]. Although many mechanical and traumatic injuries are characterized predominantly by necrotic cell death, it is now recognized that apoptosis constitutes an important part of many pathologic and physiologic processes including heat-induced injury, ischemia, organ development, and tissue remodeling [4–6]. Because many of these processes are a fundamental part of wound healing, we speculate that both necrosis and apoptosis may play an important role in wound healing after closure by laser welding. Therefore, necrosis and apoptosis were studied together in the evaluation of the rate of cell death associated with laser welding.

The objective of the present investigation was to evaluate wound strength and tissue injury in a porcine model of skin wounds. In the present study, we compared both tensile strength and tissue injury by using laser welding in the presence or absence of albumin solder. We used TCPC at different temperatures and examined the wounds at 0, 3, 8, and 14 days.

MATERIALS AND METHODS

Experimental Design

Four male Yucatan swine (12–15 kg) were used in this study. In each animal, TCPC repairs were performed at 65°C, 75°C, 85°C, and 95°C to evaluate the effects of tissue surface temperature, and repairs were performed with and without the application of a 50% human albumin solder. Tissue was retrieved for analysis at 0, 3, 8, and 14 days. Controls consisted of incisions in the day 0 animal that were sutured but without the application of solder and were not laser welded.

Under each category, a minimum of four incisions was obtained. Whereas skin wounds from all time points were subjected to wound strength testing, only the wounds from day 0 were evaluated for necrosis and apoptotic cell death. Cell death analysis was limited to day 0 wounds to evaluate the immediate effects of the laser energy on the incisions without the confounding effects of the subsequent wound-healing process.

Wound Incision and Closure

Under general anesthesia, 33 2-cm full-thickness skin incisions were made on the dorsum of each pig. The locations of the incisions were determined by using a flexible plastic template to allow reproducible wounds to be made in each animal. The incisions were closed by using a TCPC. The laser was applied as a continuous wave by using a 400-μm fiber. The power was adjusted through use of the TCPC and ranged from 1.5 to 3 W. The TCPC system consists of three parts: (a) a 1.32 μm Nd:YAG laser (Premier Laser Systems, Irvine, CA), (b) an infrared temperature-control system for tissue surface temperature measurements (Abiomed R&D, Danvers, MA), and (c) a microprocessor-based system for temperature data acquisition and real-time laser power control to maintain a constant surface temperature (Abiomed R&D). The 1.32-μm laser was used because it is moderately well absorbed by water and provides the depth of penetration necessary to perform these repairs in pig skin. On those incisions where albumin was applied, approximately 0.2 cc of 50% human albumin solder was used.

Tissue Harvest

At the time of tissue harvest, the animals were anesthetized. The dorsal skin was excised as a single flap, which included all of the incision sites. The flap was placed on a moist towel to prevent desiccation prior to the tissue analysis. Animals were killed by lethal injection of Beuthanasia-D (0.1 cc/kg). All experiments were performed in accordance with protocols approved by the Institutional Animal Review Committee at Children's Hospital, Boston.

Tissue Injury Analysis

Formalin-fixed specimens were embedded in paraffin and sectioned at 5 μm thickness. For the evaluation of apoptotic activity, the tissue was stained with terminal transferase biotin-16-dUTP nick end labeling (TUNEL). For the evaluation of cellular necrosis, necrotic zones were determined based on the characteristic morphologic features recognizable on both hematoxylin and eosin (H&E)-stained sections and TUNEL-stained sections.

Apoptosis. The tissue sections were deparafinized through xylene and ethanol and treated for 10 min in 10 mg/ml of proteinase K, buffered in 10 mM Tris 20 mM ethylene-diaminetetraacetic acid. Incubation in terminal transferase and biotin-16-dUTP was carried out for 60 min at 37°C with the addition of a streptavidine-alkaline phosphate-labeling agent. The TUNEL signal was developed with Fast Red substrate with levamisole added and counterstained with Gill's hematoxylin. Positive nuclear staining indicates the presence of nuclear DNA fragmentation. To evaluate the rate of cell death as a function of the distance from the wound edge, the proportion of apoptotic cells was determined at two locations for each half of a wound: at 0–0.5 mm from the incision and at 0.5–1.0 mm from the incision.

Necrosis. The zone of necrosis was consistently found to be an inverted cone shape. Thus, the extent of necrotic injury was quantified by measuring the maximum radius of the necrotic cones.

Wound Strength Analysis

Tensile strength measurements were performed by using a commercial tensiometer (Model Mini 55, Instron Corp., Canton, MA). All specimens were trimmed to exactly 1 cm wide and were stressed to failure at a rate of 20 mm/min. The maximum stress (kPa) needed to break the 1-cm-wide repair was determined for all specimens.

Statistical Analysis

Statistical analysis of the data was performed by using analysis of variance. Statistical significance was defined as $P < 0.05$.

RESULTS

Apoptosis

For both plain incisions and incisions laser welded with solder, the proportion of apoptotic cells (5%) seen after laser welding at temperatures of 65°C and 75°C was identical when compared with that of controls (not shown). In contrast, the rate of apoptotic cell death increased at tissue temperatures of 85°C and 95°C, which is significantly higher than the rate in controls and the tissue welded at lower temperatures ($P < 0.05$; Fig. 1). Whereas the rate of apoptosis was similar whether the wounds were repaired with or without solder at 65°C and 75°C, repairs with solder at 85°C and 95°C were associated with significantly decreased apoptotic cell death as opposed to wounds laser welded without solder (Fig. 1).

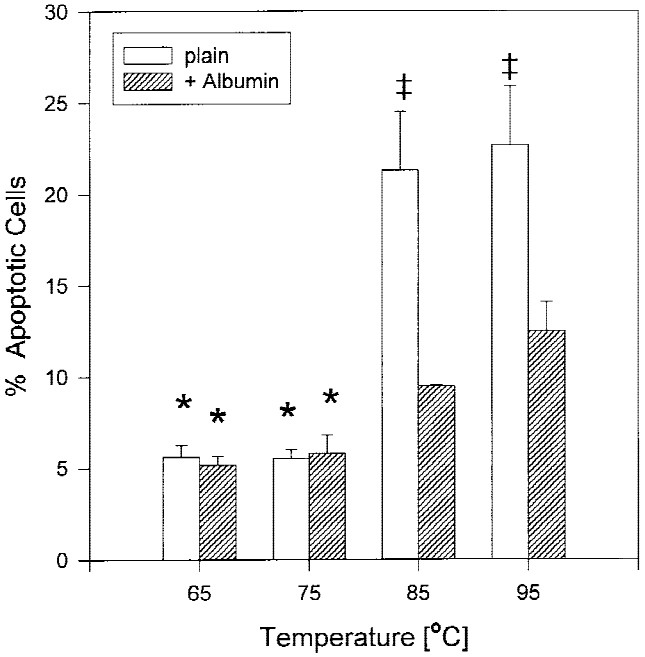

Fig. 1. Percentage of cells undergoing apoptosis in the zone 0.0–0.5 mm from the incision. Apoptosis was determined by the TUNEL method, as described in Materials and Methods. *Wounds repaired at 65°C or 75°C showed significantly less ($P < 0.05$) apoptosis than did those wounds repaired at 95°C when using either welding technique. ‡Wounds repaired with laser alone at either 85°C or 95°C showed significantly more ($P < 0.05$) apoptosis than did those repaired with albumin at the same temperatures.

At 0.5–1.0 mm from the incision edge, the rate of apoptosis was not statistically significantly different from either the plain or the solder group, regardless of the temperature used in the repairs (data not shown). Any increase in apoptosis observed at 0.5–1.0 mm from the incision edge was sporadic and minimal in extent, and no discernible effects were noted beyond 1.0 mm from the incision edge.

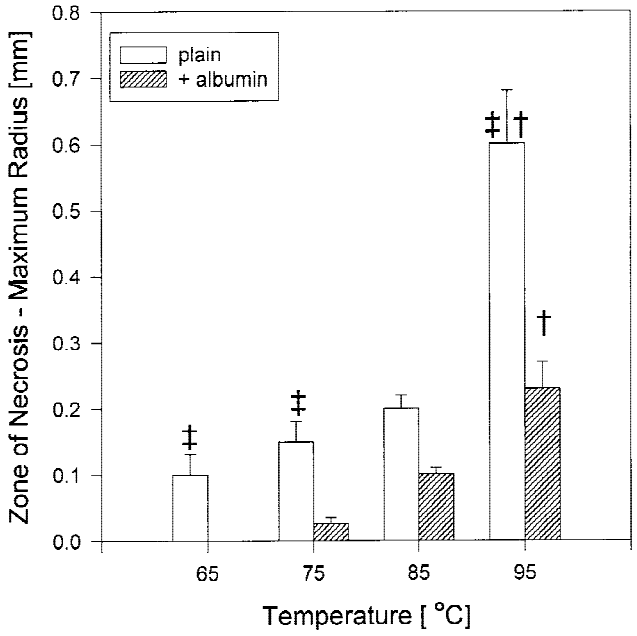

Fig. 2. Zone of necrosis in wounds repaired with or without albumin solder. The zone of necrosis was determined as described in Materials and Methods. †The maximum radius of the zone of necrosis of wounds repaired at 95°C was significantly higher ($P < 0.05$) than the radius of wounds repaired at 65°C, 75°C, or 85°C with either repair technique. ‡The rate of necrosis of plain incisions was compared with that of incisions repaired with albumin solder within the same group at a given temperature ($P < 0.05$).

Necrosis

For plain incisions and incisions laser welded with solder, the maximum necrotic zone radius was significantly greater at a tissue temperature of 95°C than at temperatures of 65–85°C (Fig. 2). Although both plain and solder-repaired wounds followed the general trend in which increasing temperatures were associated with proportionally higher rates of necrosis, the use of albumin solder was consistently observed to result in a lower rate of necrosis than in wounds closed without solder. These differences were statistically significant for the temperatures 65°C, 75°C, and 95°C (Fig. 2).

Tensile Strength of Welded Tissues

For the wounds repaired without solder, at day 0 the 95°C repairs were significantly stronger than those performed at temperatures lower than 85°C (Fig. 3A). Wounds repaired with albumin solder demonstrated a similar trend at day 0, when the repairs performed at 85°C and 95°C were significantly stronger than those repaired at 65°C and 75°C (Fig. 3A). At days 3, 8, and 14, this trend reversed, and wounds repaired at higher temperatures (85°C and 95°C) were observed to have lower mean wound strengths than wounds repaired at lower temperatures (65°C and 75°C; Fig. 3B). Although these trends were consistently observed at days 3, 8, and 14, the differences in wound strength were not statistically significant, except in the solder group on day 3, when the 95°C repair was significantly weaker than repairs at lower temperatures.

The addition of solder to laser wound closure was consistently associated with higher mean wound strengths than were wounds closed without solder (Fig. 3B). These differences were statistically significant for the day 0 wounds closed at all temperatures (Fig. 3A) but failed to reach statistical significance on the day 8 observations.

DISCUSSION

As the technology of laser tissue welding evolves, the optimal operating parameters for maximizing wound strength and healing will be refined. Our study investigated the role of tissue temperature and albumin solder on apoptotic and necrotic cell death and their relationship to wound strength and healing. Evaluation of the effects of tissue temperatures induced by laser energy in this study was made possible by the use of the TCPC system, where tissue temperatures were tightly regulated within the desired range during the tissue welding process. The two cell death processes, necrosis and apoptosis, were distinguished from each other based on staining and histologic criteria in the tissue specimens examined. Standard H&E staining characteristics were used for identification of necrosis, and the TUNEL stain was used to identify apoptotic nuclei with its characteristic DNA fragmentation.

When wound healing was studied at temperatures 65–95°C with the TCPC system, cell death by both apoptosis and necrosis was observed in different degrees in all tissue specimens examined. Differences observed in the degrees of cell death were found to be related to two parameters: (a) tissue temperature used at the time of laser welding and (b) whether 50% human albumin tissue solder was used. Although there was essentially no difference in cell death in wounds repaired at 65°C or 75°C compared with controls, tissue temperatures of 85°C and 95°C were generally associated with significantly increased apoptotic and necrotic cell death. Although some degree of tissue injury was observed at all temperatures studied, the higher welding temperatures (85°C and 95°C) appeared to generate a

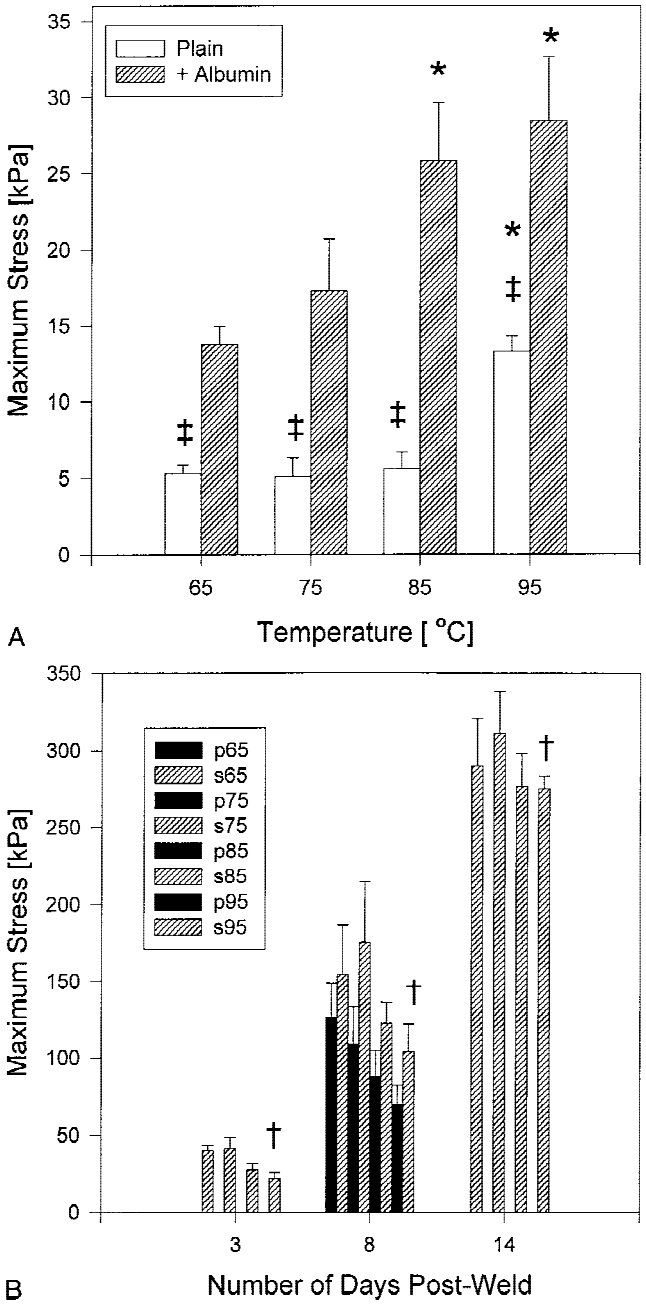

Fig. 3. Tensile strength of wounds repaired with or without albumin solder. Wound strength was determined by using a tensiometer, as described in Materials and Methods. **A:** Tensile strength of wounds repaired with or without albumin solder; results are from day 0. *Tensile strengths of wounds repaired at 85°C or 95°C were compared with those repaired at 65°C or 75°C within the subgroup by using the same repair technique (plain or with solder; $P < 0.05$). ‡Tensile strengths of plain incisions were compared with those repaired with albumin solder within the same subgroup at a given temperature ($P < 0.05$). **B:** Tensile strength of wounds repaired with or without albumin solder; results are from days 0, 3, 8, and 14. †Tensile strength of wounds repaired with albumin solder at 95°C was significantly ($P < 0.05$) weaker than that of wounds repaired at 65°C, 75°C, or 85°C. Solid bars, plain; hatched bars, albumin solder.

more severe degree of tissue injury than did the lower welding temperatures (65°C and 75°C).

The use of 50% human albumin tissue solder was associated with a lesser degree of both apoptotic and necrotic cell death, and the differences between the wound closed with and that closed without solder was especially pronounced at the higher welding temperatures (85°C and 95°C). Thus, the albumin solder appeared to have a protective effect against cellular injury. This protective effect was particularly prominent at the higher welding temperatures.

In analyzing the tensile wound strength of the laser-welded specimens at 0, 3, 8, and 14 days after welding, the welding conditions that promoted a lesser degree of cellular injury were found to correlate with superior wound strengths at 3 days and beyond after welding. In other words, the use of lower welding temperatures (65° and 75°C) and the use of 50% human albumin tissue solder both were associated with superior wound strengths at 3 days and beyond. These results are similar to those of Marinot et al. [7] who found an optimal temperature of 77°C for performing vessel anastomoses.

The day 0 data were exceptions to this rule. The higher the welding temperature, the stronger were the wounds at day 0; but the wounds welded with solder were still stronger than those welded without solder, even at day 0. We speculate that this increased wound strength observed with higher welding temperatures at day 0 was a result of tissue denaturation. However, the minimal benefit gained in wound strength immediately after welding as a result of tissue denaturation was quickly eliminated by the associated increase in tissue injury, resulting in inferior longer-term wound strengths. Thus, it would appear that optimal day 0 wound strength should not be achieved with the use of high welding temperatures, which are associated with long-term drawbacks, but rather by the appropriate use of an effective tissue solder.

The cell death after tissue welding was observed over a very short distance. Close to the wound edge, which was close to the laser beam during the welding procedure, necrosis was the most prominent finding. As the distance increased from the wound edge, where the tissue was progressively more removed from the direct path of the laser energy, the predominantly necrotic area transitioned gradually to apoptosis. Beyond a short distance of only 0.5 mm from the wound edge, no significant increase in cell death was discernible. These data demonstrate the

highly focal nature of the laser energy when applied in laser welding.

The histologic appearance of the necrotic areas and the apoptotic cells deserves further discussion. Nuclear DNA fragmentation occurs relatively early in the process of apoptosis, preceding significant disruption of the nuclear membrane; it is a relatively late event in necrosis, occurring when there is already severe nuclear and/or cellular membrane disruption. The TUNEL stain labels fragmented DNA and in itself is not specific for either apoptosis or necrosis [8]. Therefore, the presence of apoptotic cell death was determined in this study by identifying cells stained by TUNEL and that have an intact nuclear membrane. In contrast, necrotic tissue stained positively in the TUNEL assay and displayed a disrupted nuclear membrane. The use of TUNEL to identify fragmented DNA, in conjunction with H&E staining of the nuclear membrane, provides strong evidence that necrosis and apoptsis can be reliably distinguished, allowing for the separate analysis of each process, as performed in this study.

In this study, tissue injury and tensile strength after laser welding were monitored. Based on the present data, the optimal conditions for photothermal skin closure would include the use of tissue welding temperatures of 65°C or 75°C as applied by the TCPC, with the application of 50% human albumin solder. These conditions minimized immediate tissue injury and in turn were associated with superior short-term wound strengths.

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